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STUDIES ON BANANA ANTHRACNOSE

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STUDIES ON BANANA ANTHRACNOSE

By RAFAEL A. TORO, Assistant Pathologist

(With 4 plates and 1 figure in the text.)

INTRODUCTION

Banana anthracnose is known to be caused by the fungous parasite *Glœosporium Musarum* Cooke and Massee, one of the Melanconiales of the classification of Lindau placed under the Melanconiaceæ-Hyalosporæ, in Engler und Prantl's *Die Natürlichen Pflanzenfamilien* (18). The fungus was originally described from Brisbane, Australia, in 1873 and was recorded by Saccardo (31) in 1892. It was reported on fruit in the United States markets by Halsted (20) in 1892. The first record of its presence in Porto Rico is by Stevenson (38) in 1918 but it was probably introduced with the early importation of banana plants. A variety of this fungus, *importatum* was described by Laubert (29) in 1910 and is also recorded by Saccardo (32). The disease is known by various other names, as black rot, ripe-fruit rot, etc., but since anthracnose is a more broadly used term for those diseases caused by members of the genera Glœosporium and Colletotrichum, the writer has chosen the latter name as more acceptable, and besides, ripe or black rots may be caused by fungi other than *Glœosporium Musarum* Cooke and Massee.

In Porto Rico the damages caused by the fungus are restricted to market and shipping troubles, thus reducing the keeping qualities of the fruits and making them undesirable for both local and northern markets. In this respect the fungus partakes of the nature of a saprophyte. In some of the banana-producing countries, however, the losses extend to green bananas in the field, as reported by Agati (1); thus behaving as a parasite. In Porto Rico no case has been seen by the writer in which the fungus attacks green bananas in the field.

Since the fungus is found in all parts of the world where bananas are grown, either on the growing crop or on the market; and also on fruit shipped to other countries, the writer has con-

sidered it desirable to pursue a study of the morphology and physiology of the fungus, its variability, and its relation to different hosts, and the possible development of successful methods for its prevention and control. The results are here presented as a contribution to our knowledge of the parasitism and physiology of the fungus.

THE DISEASE

The first manifestations of the disease appear with the ripening of the fruit after cutting. It starts as very small dark spots which enlarge by the discoloration of the diseased tissue following the penetration of the host by the fungous hyphæ. Several spots coalesce, forming large irregular blotches with sunken centers. Sometimes the number of coalescing spots is so great that the entire fruit may be blackened. All over the surface of the blackened tissue, there appears a roseate, moistened tint which on examination under the microscope is found to be composed of the spores of the fungus.

Not all varieties of bananas show the same symptoms, and therefore, a separate description of the disease for those varieties, which show a wide range of differentiation in their behavior toward the fungus, is considered to be of importance.

APPEARANCE OF THE DISEASE ON FRUITS OF DIFFERENT VARIETIES

González (19) in his work on the cultivation of bananas in Porto Rico refers the edible varieties of the genus Musa to the species *M. paradisiaca* and *M. Cavendishii; M. paradisiaca* comprising the subspecies *sapientum* whose fruits are eaten after they are mature; and *normalis* those varieties which are cooked before eating. *M. Cavendishii* is the dwarf variety. Since González's paper is the only one on the subject of varieties in Porto Rico, varietal descriptions in this paper are in accordance with his work.

MUSA PARADISIACA SAPIENTUM

Fig or niño variety.—Spots at first small, 1 mm. in diameter, slightly sunken, numerous, later forming a blotch by the coalescing of several spots, color from brick red to madder brown.[1] Spore masses very seldom found in nature, but usually developed in moist chamber.

Giant or "gigante" variety.—Small, roundish, sunken, numerous

[1] Colors as given correspond to Ridgway's "Color Standards and Nomenclature."

dusky drab to blackish brown spots appear first. These increase in size sometimes coalescing to form streaks covering the entire surface of the fruit. (Plate I, fig. 2.) Fruiting bodies of the fungus appeared in from three to five days after infection, as pinkish sticky masses which increased in size by coalescing. Around some small spots a circular zone of ashy aerial mycelium is formed, if the banana is kept in moist condition. Finally the whole surface is blackened, the interior tissues are softened by the penetration of the fungous hyphæ, become bitter, and the ultimate destruction of the fruit takes place.

Apple or Manzano variety.—This variety shows a certain degree of resistance to the disease. Spots are fewer in number, less definitely margined, slightly sunken, 1/32–7/24 inch long, 1/18–5/24 inch wide, fucous black on the margins, tawny olive in center. Spore masses small, pink and few in number.

MUSA PARADISIACA NORMALIS

The varieties of this sub-species show a greater degree of resistance than those of the sub-species just mentioned.

Chamaluco. — The disease begins by forming blackish brown streaks and later developing, scattered, shrimp-pink masses of spores. Around some irregularly formed spots, a weft of whitish-pink mycelial threads is formed, inclosing the spore masses. Diseased tissue is more abundant near the ends of the fruit, where some injury always takes place on account of careless handling.

Plátano.—This is the most resistant variety found. Even if an abrasion occurs on the peel while the fruit is maturing no signs of the disease are seen until maturity is well advanced. It is then that the fruit gradually turns blackish brown until the whole surface may be discolored. Fruiting bodies very seldom appear unless it is kept in a moist place. These are manifested in the form of very small, dry, pink masses, with a mycelial weft covering them. The dryness of the spore masses is a striking characteristic in contrast with the moist condition in the other varieties.

MUSA CAVENDISHII

Enano or dwarf.—This is the most susceptible of all the varieties studied. Once the fungus has entered the epidermal tissue, the destruction of the fruit takes place more rapidly than in any other variety. The symptoms very much resemble those of the Giant. (Plate I, fig. 3.)

THE FUNGUS

There has been observed a great difference in the pathogenicity of this fungus as regards its attacks in other countries as reported by Agati (1) Cobb (9) and others. Whether this variation is due to a difference in virility of the fungus in the countries where it is found, or to some other factor, is an unsolved problem; but it can be safely said that it may be due to different strains of the fungus which have a wide range of virility.

That there exist widely distinct strains within species of the same fungus, which are more or less virulent, has been demonstrated by Jennings (25), Hansen (22), Barber (3), Burger (7), La Rue and Barlett (27), Christensen (8), Leach (30) and others. Cultural studies of *Glœosporium Musarum* Cooke and Massee, made from the different varieties of bananas previously mentioned and on different media, show that there are strains of the fungus which behave differently among themselves when treated under similar conditions. These results will be shown later.

Burger (1. c.) attributes the difference in pathogenicity of *Colletotrichum glœosporioides* Penz. in California and Florida, to a difference in the amount of rainfall in these two States.

Barrus (4) at first reported certain varieties of beans as resistant to the anthracnose fungus *Colletotrichum lindemuthianum* (Sacc. & Magn.) Brios. & Cav. But later he (5) found that when other strains of the fungus were used these varieties became susceptible to the disease.

Not only the variations in weather conditions and virility of strains have been considered when solving the problems of disease resistance in plants; but the anatomical and physiological characters of the host have been considered as well. Hawkins and Harvey (24) have proved that the resistance of a variety of potato to *Pythium de Baryanum* Hesse was due to the thickness of the cell walls of the tuber. Cook and Taubenhaus (10) (11) made studies indicating that disease resistance in plants may be due to the high tannin content of the cell sap which was toxic to the fungi studied or to the presence of an enzyme capable of forming a tannin-like body which is also toxic to the fungi. Some studies were also conducted by the writer to determine whether any of the above-mentioned factors are of any importance in the partial resistance or susceptibility of the varieties of banana and the results will be shown in a latter paragraph.

MORPHOLOGY OF THE BIOLOGIC FORMS

That the biologic forms of the anthracnose fungus of the banana can be distinguished on the basis of spore size may be easily seen from a study of Tables I, II and III. Stackman and Piemeisel (37) have shown that the biologic forms of *Puccinia graminis* Eriks. & Hern. can be distinguished on the basis of spore size. La Rue and Barlett (27) also distinguished strains on the basis of spore size within the species *Pestalozzia Guepini* Desm. Burger (7) with *Colletotrichum glœosporioides* Penz., Leach (30) with *Colletotrichum lindemuthianum* (Sacc. & Magn.) Brios. & Cav. and Christensen (8) with *Helminthosporium sativum* Pammel, King and Bakke, also found that biologic forms of each of the fungi studied can be distinguished on the basis of spore sizes.

A series of spore measurements were made of each of the strains studied. In every case one hundred spores were measured for length and one hundred for width and the results are tabulated in Tables I, II and III.

TABLE 1

Spore lengths of different biologic forms of *Gloeosporium Musarum* (in microns)

Strain	Variety	Place	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	Range	Mode
I	Dwarf	Las Marías						31	15	28	6	2	4	5	4	3	2	15-24	15
II	Dwarf	Lares		16	8	0	36	20	8	12								11-17	14
III	White	Río Piedras						15	11	52	9	3	6	4				15-21	17
IV	Dwarf	Río Piedras		40	9	3	19	7	8	10	4							11-18	11
V	Plantain	Las Marías						24	11	47	18							14-17	16
VI	Red	Río Piedras						18	25	12	16	12	5	6	6			14-21	15
VII	Fig	Ponce						12	24	12	11	8	10	14	4	5		14-22	15
VIII	Dwarf	Río Piedras					9	6	48	5	10	9	5	8				13-20	15
IX	Apple	Las Marías			9	10	21	35	7	13	5							12-18	15
X	Giant	Las Marías	7	30	20	43												10-13	13
XI	Chamaluco	Río Piedras		18	20	14	40	8										11-15	14

TABLE II

Spore widths of the different biologic forms of *Gloeosporium Musarum* (in microns)

Strain	3	3.5	4	4.5	5	5.5	6	Range	Mode
I			15	48	20	9	8	4-6	4.5
II	28	21	51					3-4	4
III		18	40	20	12	10		3.5-5.5	4
IV		23	30	35	12			3.5-5	4.5
V	21	58	12	9				3-4.5	3.5
VI		21	36	18	15	10		3.5-5.5	4
VII		28	50	12	8	2		3.5-5.5	4
VIII		22	41	20	8	6	2	3.5-6	4
IX	10	39	26	25				3-4.5	3.5
X	28	52	15	5				3-4.5	3.5
XI	15	36	38	11				3-4.5	3.5

Table I summarizes the length of one hundred spores for each of the eleven biologic forms of *Glœosporium Musarum* Cooke & Massee encountered on some of the different banana varieties. These varieties were secured from widely separated localities throughout the island. As demonstrated in the table, strains from humid places do not differ much in their range of variation from those from dry localities. This indicates that the substratum is a factor of much more importance in determining spore size than weather conditions. Table II summarizes the width of the one hundred spores of each of the biologic forms measured in Table I for length. In Table III the biometrical constants with their probable errors for the lengths of each of the strains measured on Table I, is computed.

TABLE III

Calculated biometric constants for length of the different strains of *Gloeosporium Musarum* (in microns)

Strain	Mean	Standard deviation	Coefficient of variability
I	17.36 ± .155	2.3 ± .11	13.22 ± .65%
II	14.06 ± .129	1.92 ± .09	13.65 ± .65%
III	17.32 ± .095	1.41 ± .06	8.14 ± .11%
IV	13.87 ± .12	1.78 ± .08	12.83 ± .61%
V	15.80 ± .06	.95 ± .04	6.00 ± .28%
VI	16.46 ± .14	2.12 ± .10	12.00 ± .57%
VII	17.16 ± .19	2.82 ± .13	16.43 ± .79%
VIII	16.10 ± .11	1.74 ± .08	10.80 ± .52%
IX	14.72 ± .10	1.52 ± .07	10.30 ± .49%
X	11.76 ± .06	.96 ± .04	8.18 ± .39%
XI	12.90 ± .08	1.21 ± .05	9.30 ± .43%

From an examination of Tables I and II it can be easily seen that some strains depart from the original description of the fungus given by Cooke (12) as much as 12 microns for length and 1 micron for width. Laubert (29) established his variety *importatum* on this difference in spore measurement; but Stoneman (39), Shear and Wood (34), Lasnier (28), Delacroix (13), Voglino (41) and Savelli (33), who made studies with this fungus, also found their measurements to be extremely variable from the original measurements and did not make any attempts to establish new varieties. In this connection Delacroix says:

"Malgré ces différences, et bien que je sois sur des mensurations que je fournais, je ne crois pas qu'il y ait bien de faire une spéce distincte."

While attempting to make cultural studies of the different biologic forms it was found that the modal length for some of the strains when grown on culture medium, varies much. Moreover,

sometimes the conditions were reversed in such a way that a strain with a high modal length in nature gave rise to a smaller modal length when grown in culture medium, and conversely. This condition was not always true, as it was very soon learned that the size and form of the spores were markedly influenced by the physical and chemical composition of the culture media. This condition is graphically illustrated for strain X in figure I.

PHYSIOLOGY

(1) Effect of the Medium. — The cultural characters of various organisms, have been used to distinguish between species or forms which can not be readily differentiated on morphological basis. Appel and Wollenweber (2) and Sherbakoff (36) have demonstrated the value of this method for species of Fusaria. Thom (40) made cultural studies with species of Penicillium and also demonstrated the value of this method. It was thought, therefore, that under the uniform temperature conditions prevailing in tropical climates, cultural studies to differentiate between the biologic forms of *Glœosporium Musarum* Cooke and Massee, could be made to advantage.

The method used for isolating spores and starting pedigree cultures, resemble somewhat that used by Kauffman (26). Cultures were started from conidia developed in the ripe bananas which were kept in moist chambers. The spore masses were diluted in water blanks and then sprinkled over the surface of cooled culture medium. The medium was first poured thinly into petri dishes with thin bottoms and allowed to cool. After 6 to 10 hours, the time required for starting germination, the germinating spores were located with the microscope by examining the surface of the agar with the cover removed. Transfers of single spores were then made to tubes of the same medium.

The media used were Cook's No. II, corn meal, oat meal, banana and potato agar. Cook's No. II medium is prepared as follows: to 500 cc. distilled water add 10 gr. agar-agar, 10 gr. dextrose and 5 gr. peptone, .25 gr. dipotasium phosphate and .25 gr. magnesium sulphate. Boil, filter, tube and autoclave for twenty minutes at 15 lbs. pressure. The agar content of the medium was modified and put a little higher than usual because of the higher tropical temperature, the proportion given by Cook (11) being 7½ gr. agar-agar. Banana medium is prepared as follows: Take 50 gr. ripe peeled bananas and boil in 500 cc. distilled water. Press through cheese-cloth, add 10 gr. agar-agar, boil again, filter, tube

and autoclave for 20 minutes at 15 pounds pressure. Corn meal, oatmeal and potato media were prepared in the usual manner.

All media was titrated to a point plus 3 according to Fuller's modified method as directed by Duggar (15). In all cases, care was taken to use, approximately, the same amount of media.

It was found that with Cook's No. II medium the most striking differentiation between the different biologic forms of *Glœosporium Musarum* Cooke and Massee, was obtained. Therefore, this medium was selected for my cultural studies. With the other media, however, very close resemblances were found between some of the strains while other strains varied greatly in duplicate cultures of the same medium; a fact also noted by Christensen (8) on *Helminthosporium sativum,* Pamnel, King and Bakke.

For convenience in differentiating between the biologic forms of *Glœosporium Musarum* Cooke and Massee, as they grow on Cook's No. II medium, the following key was prepared. With the aid of the key, it is not difficult to see how one strain differs from any one of the others. Moreover, a new strain can be identified after growing it for some time on this medium.

KEY OF STRAINS

Description of biologic forms of *Glœosporium Musarum* Cooke & Massee as shown by cultural characteristics on Cook's No. II agar:

Spore masses minute, zonnate, ochraceous salmon, aerial mycelium white on outside, from dwarf variety ---------- I
Spore masses few, light ochraceous salmon to black, covered with white mycelium; from dwarf variety ---------- II
Spore masses none, spores borne in a hyphomycetous fashion, culture then strawberry pink, otherwise white; scant, from white variety ---------- III
Mycelium profuse, white, shrimp-pink on sporulation, spores borne in a hyphomycetous fashion, from dwarf ---------- IV
Mycelium scant, spores abundant, shrimp-pink in mass, no acervuli, sporodochia present, from Plantain ---------- V
Aerial mycelium white, profuse, spore masses La France pink, acervuli none, sporodochia present, from Red ---------- VI
Aerial mycelium white, scant, spore masses few, Hermosa pink, from fig- VII
No mycelium seen, whole culture a mash of marsh yellow spore masses, from dwarf ---------- VIII
Sporodochia shrimp-pink, aerial mycelium scanty, from Apple ---------- IX
Mycelium few, spore masses zinc-orange, numerous, from Giant ---------- X
Aerial mycelium white, forming a circle around the inconspicuous spore masses, spore masses very light safrano pink, from Chamaluco ---------- XI

From the above data, it can be seen that each biologic form,

behaves differently from the others; at least when grown on Cook's No. II agar medium; these differences being sufficient to justify their differentiation on their cultural characteristics.

In one of the cultures setæ were found (Plate IV, fig. 14), but all attempts to secure these again in subsequent cultures resulted in failure. Stoneman (39), Edgerton (16) and Shear and Wood (34) while working with some anthracnose producing fungi came to the conclusion that setæ are variable as to presence or absence and that they are not reliable morphological characters to use in separating genera. Stoneman and Shear and Wood did not find setæ in any cultures of *Glœosporium Musarum* Cooke and Massee. Barrus (6) states that cultural work with *Colletotrichum lindemuthianum* (Sacc. & Magn.) Brio. & Cav. has demonstrated that the presence or absence of setæ, is not a character of sufficient stability to serve as a basis for determination of generic position.

Lasnier (28), Shear and Wood (34), Stoneman (39) and Savelli (33) made cultural studies of *Glœosporium Musarum* Cooke and Massee. Lasnier and Savelli found great variations on the method of spore production. They found fruiting bodies resembling pycnidia, in this respect giving to the culture a character of the Sphæropsidales. Acervuli were formed on some of Lasnier's cultures, but none on Savelli's. Not only were these two methods of spore bearing found but the spores were also produced on conidiophores and discharged without any definite body formed, thus giving a character of the Moniliales.

Spores were found by the writer to be produced the second day after sowing on the culture media. At first, the conidia are produced at the end of the hyphal threads. It was observed that there is a constriction formed at the end of the hypha, and the spore thus formed, pushed aside; the hypha elongates then to about the same point, when a constriction again occurs (Plate IV, fig. 18). Hence, several spores are formed, from a single branch and these can be seen lying side by side at the end of the branch. In some cultures, and always with particular strains, this condition of spore formation remained constant throughout, thus giving to the fungus a character of the hyphomycetes (Plate IV, fig. 15). In other cultures, however, although the spores are freely formed at first at the end of the conidiophores, soon the radiating mycelium becomes dotted with pink, small bodies, which when examined proved to be small Sporodochia (Plate IV, fig. 16). These Sporodochia increase in size sometimes attaining ¼ to 1½ mm. in di-

ameter, by the crowding together of several masses of spores. They are covered by a gelatinous matrix and seated on a pseudo-stromatic base of mycelial threads. Intermixed with the sporodochia, in some cultures, small, immersed black fruiting bodies suggesting the presence of perithecia were seen. When carefully examined under the microscope, it was found that these bodies were unostiolate, carbonaceous pycnidia (Plate IV, fig. 17). No acervuli, however, were ever formed in any of the cultures. This behavior of the fungus in culture suggests that fungi sometimes may be made to develop characters very distinct from those usually attained in nature, and proved conclusively that no description of a fungus is complete unless it is made from all possible angles by which the fungus is question may be made to vary.

SPORE GERMINATION

The process of spore germination of various fungi of the genera Glœosporium and Colletotrichum, has been studied by Halsted (21), Hasselbring (23), Edgerton (17) and others. Stoneman (39) found that *Glœosporium Musarum* Cooke and Massee germinates by the formation of one or two germ tubes originating at or near the end of the conidium and that the conidium remained single-celled on germination. The writer found that, although the majority of the spores remained single-celled while germinating on culture media, there were, however, some two-celled spores; and moreover, in water cultures, on the glass slide nearly all the spores became two-celled.

The first sign of germination in water is a slight swelling of the spore, which is followed shortly by the protruding of a germ tube from any part of the spore, usually the end. (Plate IV, fig. 20). If the germ tube continues increasing in size it gives rise to secondary hyphal threads; but if the germ tubes come in contact with the hard surface of the slide or the cover glass, a thick-walled, roundish, brownish structure is formed at the place of contact (Plate IV, fig. 19). This structure, called appresorium, was also observed by Halsted (21), while studying other anthracnose producing fungi, and he suggested that they may be formed as a protective body to carry the germinating spore over unfavorable periods. He attributes some taxonomic value to them. Hasselbring (23) from studies on *Glœosporium fructigenum* Berk, believed them to be adhesion organs during early stages of infection and thinks they are formed as a result of contact stimuli but lose this power

of reacting in culture media. He finds the appresoria more resistant to unfavorable conditions than the spore. Dey (14) in his studies with *Colletotrichum lindemuthianum* (Sacc. & Magn.) Briso. & Cav. found that appresoria are formed whenever the germ tube comes in contact with a hard substance which acts as a stimulus for its formation.

In culture media, the writer found that few and sometimes no appresoria are formed and that the spores very seldom become two-celled in germinating. There was observed, however, the slight swelling of the spore just before the protruding of the germ tube, a fact also found by Edgerton (17) in *Colletotrichum lindemuthianum* (Sacc. & Magn.) Brios. & Cav.

In an endeavor to determine to what extent the percentage of spore germination was influenced by different media, it was found that distilled water gave the lowest percentage of germination—3 to 6 per cent. With Cook's No. II medium the higher percentage of germination was obtained—95 per cent. Between these two media, gradations in percentage-germination occurred as shown by Table IV. It was also found that with distilled water the time for germination is lengthened to as much as 20 hours, while with banana agar, complete germination was attained in six hours.

TABLE IV

Comparison of percentage germination of spores between ordinary media and media to which a piece of host tissue was added and time requirement for germination.

Medium	Per cent of germination		Time required to start germination (in hours)	
	added No tissue	Medium plus tissue	No tissue added	Medium and tissue
Distilled water	5	80	20	18
Tap Water	20	85	17	14
Cook No. II. Agar	95	98	6	4
Corn Meal Agar	80	95	8	6
Potato Agar	35	85	8	6
Banana Agar	95	95	6	6
Oats Agar	95	95	8	6

When a piece of host tissue was placed on the culture medium, it was found that the germ-tubes of all spores in an area of 30 millimeters from the host tissue, turn in the direction of the tissue. This shows that chemotropic response is manifested under the stimulus of the host tissue (Plate IV, fig. 21).

HOST RELATIONSHIP

Various investigators, among them Cobb (9), Shear and Wood (34) and Laubert (29), have made cross-inoculation studies with *Glœosporium Musarum* Cke. & Massee on different hosts, and they all have succeeded, to a greater or less degree, in producing a diseased condition on the hosts inoculated.

The writer made inoculation studies on the fruits of tomatoes, níspero (*Achras zapota*), guavas, green dwarf bananas and cowpeas in the field. The studies were repeated with the avocado and mango fruits in the laboratory in a moist chamber. Field inoculation experiments were also done on avocado leaves and trunks. Other workers also did inoculation experiments under laboratory conditions. In their experiments, the fruits were removed from the plant, disinfected, rinsed with distilled water and placed in a moist chamber. The results obtained have not been accepted as conclusive by some investigators. In 1898, Miss Stoneman (39) as a result of her comparative studies of some anthracnoses, said:

"Very little dependence can be placed upon the results obtained from cross inoculations made in the laboratory. The host to be inoculated is placed in a moist chamber or under a bell jar, where the moisture of the fruit is conserved, and the conditions are favorable for any fungus which is already lurking in the tissue to develop. On the other hand, it has been shown that the fungi of this group easily adapted themselves as saprophytes and a watery fruit like the watermelon or citron, which has been separated from the plant, has lost to a degree the power of resistance and becomes more or less of the nature of a culture medium."

Most of my studies referred above were made under field conditions. The results follow:

Avocado.—On the fruit, puncture and surface spraying inoculations were made under a bell jar in the laboratory. Three days after the puncture inoculation was made, the tissue around the wounded surface began to change in color. The discoloration was dark-green, very much darker than the usual color of the fruit. This discoloration increased throughout until the whole surface was discolored. At this time, the contents were all softened and watery, but with no bad odour. Soon after this, pink spore masses of the fungus appeared. The spores were typical of the banana. Although there was a little difference in their size and shape this change was not greater than the one expected on culture medium. With surface spraying in which the surface was uninjured, the discoloration began five days after the inoculation. This appeared

first on the wound produced in removing the fruit from the tree. The rot worked more slowly but the final symptoms were the same as with the puncture. Surface spraying of the fungus on the leaves of potted small trees gave negative results, and also inoculation of the mycelium on a wound in the bark of the stem.

Mango.—As with the avocado, surface and puncture inoculations were made on the fruit. The surface spraying gave negative results while with the puncture the results were positive. The fungus worked more slowly on the mango than on the avocado and no fruiting bodies were produced on the former. There was, however, the discoloration and softening of the surface and contents of the fruit. No inoculations were made either on the leaves or on the bark of young trees.

Tomato.—Puncture inoculations made on the fruit of the tomato while on the plant, began to show positive results three days after they were made. The first symptom is the discoloration around the wound, which gradually enlarges, covering the whole fruit. While attempting to recover the fungus again from the tomato, it was found that not only was Glœosporium present, but other fungi, principally Fusarium. Therefore, this inoculation was recorded as doubtful. Repeated inoculations on the fruits, in the field as well as in the laboratory, gave negative results. Surface spraying on the leaves, stem and fruits gave negative results.

Guava.—The guava gave positive results with both methods. The symptoms very much resemble those of *Glœosporium Psidii* Del. as described by Sheldon (35), but the conidia did not correspond to those of the guava.

Sapota.—Both methods gave negative results.

Dwarf banana.—Several experiments were tried on the field and in the laboratory to produce the disease on green bananas. All surface inoculations resulted in failure, even if they were made under laboratory conditions. With puncture inoculations success was attained only after the fruit began its process of maturation, and then it was found that not only *Glœosporium Musarum* Cke. & Massee was present on the cracked fruit, but other organisms were found as well. The series of failures to produce the disease on healthy, green bananas in the field, proves conclusively, that, so far as its pathogenicity is concerned, the fungus is at least a very weak parasite, if not a true saprophyte. No results were obtained while trying to produce the disease on the leaves and stems of the bananas. Mature sound bananas when kept in a moist cham-

ber became diseased. This suggests that the fungus may be present on the fruit, even when it is green, but that it does not manifest itself, because of some inhibitory factor.

Cowpeas.—Characteristic anthracnose lesions were produced on inoculated cowpeas. Both, puncture and surface spraying of spores gave positive results.

The experiments of the writer with *Glœosporium Musarum,* Cke. & Massee as well as those of others with some anthracnose producing fungi, have proved, that these fungi adapt themselves to such a wide range of conditions that much cultural and cross-inoculation work need be done in order to ascertain the exact relationships of each individual organism to make specific determinations more tenable.

PARASITISM

The question of disease resistance and disease susceptibility in plants has given rise to different theories and has directed the attention of many workers, toward lines of research which have brought out definite facts and have established certain principles tending to solve these problems. Although much has been learned along these lines, the question is still far from answered. Recent studies of the problem have demonstrated that true resistance must depend either on some anatomical character of the host tissue or on the physiology of its protoplasm. The literature on the subject is copious and has been reviewed by Cook and Taubenhaus (10) and others.

EFFECT OF THE FUNGUS ON THE HOST

It has been stated by Delacroix (13) and others, that *Glœosporium Musarum* Cke. & Massee is a wound parasite and that it can not cause banana anthracnose when the fruits are carefully handled. Although, it was found that a wound is one of the prevailing factors for a diseased condition, nevertheless other factors may be also involved, each of which might have a greater or less influence in creating a disease condition. Since there are many possible ways by which a pathogen can enter its host; the writer thought to study at least four of them: (1) if the penetration is mechanical; (2) if it enters through an opening already made; (3) whether there was any dissolving enzyme in the mycelium of the fungus which destroys the host tissue and; (4) if the host itself contains any toxic substance which will inhibit the fungous attack. That the fungus can not attack the green or partly matured sound ba-

nanas in the field even if the most susceptible variety was inoculated, can be seen from a study of page 19. (See experiments with inoculation in banana.) Completely matured bananas, when placed under proper conditions, however, became diseased, regardless whether injured or not. This fact has been correlated with a change in the chemical and physical composition of the peel of mature bananas. The peel then has a higher sugar content and the tissue is softened. It has been also proved, that any abrasion on the peel opens the way for the fungus to enter and cause the disease.

Now, does the question of the resistance of certain varieties of banana to the disease lie in the power of the fungous mycelium to dissolve the cutinized tissue of the susceptible hosts or does it lie on the toxicity of some substances of the host protoplasm?

Experiments were conducted to solve the problem of the mycelium enzyme and chemical analyses were made of the peel of resistant and susceptible varieties of banana. To prove whether the mycelium had any substance which would dissolve the host tissue the following experiment was made. An extract of the mycelium content of the fungus was obtained as follows: Cultures of the fungus were grown for 12 days in Cook's No. II medium to which no agar had been added. Mycelium and sporulation on this medium was profuse. The flask in which the culture was growing was emptied in a mortar, the mycelium grounded with sand, and the cell contents of the fungus extracted with distilled water. Then the extract was filtered three times. The liquid substance thus obtained was divided and put in two flasks containing equal amounts of the filtrate. One of them was boiled. Then, pieces of mature and green dwarf bananas were placed in each of the flasks containing the boiled and non-boiled mycelium extract. A check was also made with sterilized distilled water.

It was found that with the extract which was not boiled a disintegration of the parenchyma tissue of the mature banana was evident within two days. No change was noticed on the pieces of immature bananas. No effect was observed on the epidermis of both mature and immature pieces of bananas. Neither the boiled extract nor distilled water had any effect on the parenchyma tissue of the epidermis of either mature or green pieces of bananas. This effect makes clear that the ultimate destruction of the banana after inoculation is brought about by enzymic action of the fungus

but that this enzyme is not strong enough to destroy the stronger cutinized cells of the epidermis.

EFFECT OF THE HOST ON THE FUNGUS

The results of the investigations by Cook and Taubenhaus (10) on the toxicity of tannin, showed that spores of *Glœosporium Musarum* Cooke and Massee were inhibited in their germination when the culture media contained more than .6 per cent tannin. Analyses were made of the peel of green and mature bananas of three different varieties, varying in degrees of susceptibility and the results are tabulated on table V.

TABLE V

Analysis of the moisture and tannin contents of the peel of some banana varieties

Variety	Condition	% of Moisture	% of tannin in dry material
Chamaluco	Green	82.65	.0825
Chamaluco	Mature	75.11	.155
Gigante	Green	89.49	.155
Gigante	Mature	87.60	.310
Plátano	Green	71.83	.0825
Plátano	Mature	58.95	.0825

From the above data it is evident that the most susceptible variety of banana to the disease, Gigante, contains the highest percentage of tannin. Moreover, the tannin does not reach the limit within which germination takes place as found by Cook and Taubenhaus (1. c.). It was also found that the tannin content of the peel increases with maturity. All these results, bring us to the conclusion that tannin has no effect on the infection by *Glœosporium Musarum* Cke. and Massee and that resistance or susceptibility are correlated with anatomical characters of the host rather than with the physiology of its protoplasm.

PREVENTION AND CONTROL

It has been shown that *Glœosporium Musarum* Cke. & Massee, the causal organism of the anthracnose or ripe rot of the bananas, was unable to cause the disease on green uninjured bananas in the field. This fact, undoubtedly demonstrates, that care should be exercised to avoid any injuries on the peel, for once the peel is injured, it becomes a source of infection by which the fungus may gain entrance to the fruit.

Since some varieties of bananas are more susceptible to the

disease than others, the planting of the varieties most resistant to the attacks of the fungus should be encouraged and thus reduced to a minimum the losses which result from infections. A moist atmosphere with a high temperature, are also favorable for the development and infection by the fungous hyphæ. Therefore, it is important to hang the banana bunches where a current of air passes between the "hands" and so reduce the amount of moisture and lower the temperature which otherwise would result if the bananas were kept in a pile. In preparing bananas for shipping careful handling is the most important thing. They should be kept cool and ventilated until they reach their destination.

Although spraying with bordeaux mixture has been considered the most important general method of preventing diseases, it is not recommended in the case of banana anthracnose, for two outstanding reasons. In the first place, spraying will bring a discoloration to the fruits which will make them unsalable; secondly it will not pay, because the damages due to the fungus are not so severe, as to justify the use of spraying materials.

SUMMARY

1. Banana anthracnose is caused by the fungus *Glœosporium Musarum* Cooke and Massee.

2. The disease is characterized by the formation of sunken, dark blotches on the peel of ripe fruits accompanied by a moist tint of salmon color spore masses.

3. There is a great variation in the size of the spores of the fungus on different varieties of bananas, even when inoculated from the same culture.

4. The culture media influenced the size of spores of the fungus. Different biologic forms were not influenced in the same manner by the same medium. The variations in size due to the influence of the culture medium were sometimes greater or sometimes less than the difference in size of two forms on the same medium. Therefore, the influence of the medium, so far as spore sizes is concerned, is of no practical importance in separating biologic forms of *Glœosporium Musarum* Cke. & Massee on the basis of spore size.

5. The behavior in culture medium of the different forms of the fungus, however, furnished a more definite clue for separation between them. It was found that Cook's No. II medium brought

out this point more strikingly. It can be seen, that of all the cultures made from a great number of diseased fruits eleven of them showed characteristic points of such a marked difference as to justify their separation on the basis of their growth on culture medium. With this fungus the physiological reaction on the culture medium is of more taxonomic importance than the morphological character of the spores.

6. The spores of the fungus germinate by the formation of one or two germ tubes. They may become one-septate in germinating, but usually they remain single celled. They germinate quicker in culture media than in water. Appresoria were formed whenever the germ tube came in contact with the hard surface of the slide.

7. The fungus is not only able to cause a diseased condition on ripe bananas but other hosts may be attacked as well. Cowpeas, mangoes, avocados and guavas gave positive results, while nísperos gave negative results. The tomatoes gave doubtful results. It might be possible that with much cultural and cross inoculation work many of the now existing species of Glœosporium, as well as of many other fungi, may be reduced to synonymy.

8. It was found that the fungus mycelium contains a substance capable of dissolving the parenchyma tissue of fully matured bananas but which has no effect on unripened bananas nor on the epidermal cells of either mature or unmature fruits.

9. The susceptibility or resistance of bananas to the disease are not dependent on the tanning content of the peel. It is therefore possible that mechanical injury accompanied by a change in the chemical composition of the peel thus increasing the sugar content, are the prevailing factors in exposing bananas to the disease.

10. No preventive methods can be practised advantageously, except the aeration of the "hands" in the bunches to allow ventilation, reduce the moisture and insure proper temperature.

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LITERATURE CITED

1. Agati, Julián. Banana Stem and Fruit rot. The Philippine Agriculturist **10**: 411–422. 1922.
2. Appel, Otto and Wollenweber, H. W. Grundlagen einer Monographie der Gattung Fusarium (Link). Arbeiten aus der Kaiserlichen Biologischen. Anstalt für Landund Forst wirtschaft. **8**: 1–207. 1910 (Sherbakoff, C. D.) Fusaria of Potatoes, N. Y. (Cornell), Agric. Exp. Sta. Memoir **6**. 1915.
3. Barber, M. A. On heredity in certain microorganisms. Kansas Univ. Sc. Bul. **4**: 1–48. 1907.
4. Barus, Mortier, F. Varietal resistance of beans to anthracnose. (Abst.) Phyto. **1**: 107–108. 1911.
5. ——— ———. Variation of varieties of beans in their susceptibility to anthracnose. Phyto. **1**: 190–195. 1911.
6. ——— ———. Bean anthracnose. N. Y. (Cornell) Agric. Exp. Sta. Memoir **42**. 1921.
7. Burger, C. F. Variations in Colletotrichum glœosporioides. Journ. Agric. Res. **20**: 723–736. 1921.
8. Christensen, J. J. The parasitism of Helminthosporium sativum. Univ. Minn. Agric. Exp. Sta. Tech. Bul. **11**. 1922.
9. Cobb, N. A. Letters on the diseases of Plants. Second Series N. S. W. Agric. Dept. Misc. Pub. **666**: 1–27. 1904.
10. Cook, Mel. T. and Taubenhaus, J. J. The relation of Parasitic Fungi to the Contents of the Cells of the Host Plant. (I—Toxicity of Tannin). Del. Coll. Agric. Exp. Sta. Bul. **91**. 1911.
11. ——— ———. The relation of Parasitic Fungi to the Contents of the Cells of the Host Plant. (II—The toxicity of Vegetable Acids and the Oxydizing Enzyme) Del. Coll. Agric. Exp. Sta. Bul. **97**. 1912.
12. Cooke, M .C. New Australian Fungi. Grevillea **16**: 3. 1887.
13. Delacroix, G. Sur le Mode de developpment du Champignon du "Noir des Bananes." Agric. Prat. des Pays. Chauds. 89–91. 1902.
14. Dey, P. K. Studies in the physiology of parasitism. V infections by Colletotrichum lindemuthianum. Ann. Bot. **33**: 305–312. 1919.
15. Duggar, B. M. Fungous disease of Plants. Ginn. & Co. 1909.
16. Edgerton, C. W. The physiology and development of some anthracnoses Bot. Gaz. **45**: 367–408. 1908.
17. ——— ———. The bean anthracnose. La Agric. Exp. Sta. Bul. **119**. 1910.
18. Engler, A. und Prantl, K. Die natürlichen Pflanzenfamilien **1**. 1900. Melanconiales. Von G. Lindau, 398–415.
19. González, Policarpo. El Cultivo del Banano en Puerto Rico. Bul. **25**. 1920.
20. Halsted, B. D. A study of fruit decays. N. J. Agric. Exp. Sta. Ann. Rept. **13**: 322–333. 1893.

21. ——— ———. The secondary spores in anthracnoses. N. J. Agric. Exp. Sta. Ann. Rept. **13**: 303–306. 1893.

22. Hansen, E. C. Recherches sur la physiologie et la morphologie des ferments alcoholiques. Comptes Rendus des Travaus du Laboratorie de Carlsbert **5**: 1–38. 1900.

23. Hasselbring, Heinrich. The appresoria of the anthracnoses. Bot. Gaz. **42**: 135–142. 1906.

24. Hawkings, Lon A. and Harvey, Rodney, B. Physiological study of the Parasitism of Pythium debaryanum Hesse on the Potato tuber. Journ. Agric. Res. **18**: 275–292. 1919.

25. Jennings, H. S. Pure lines in the study of genetics in lower organisms. American Naturalist **45**: 79–89. 1911.

26. Kauffman, C. H. A contribution to the physiology of the Saprolegnaceæ with special reference to the variations of the sexual organs. Ann. Bot. **22**: 361–387. 1908.

27. La Rue, Carl D. and Barlett, N. H. A demonstration of numerous distinct strains within the nominal species Pestalozzia Guepini Desm. Am. Jour. Bot. **9**: 79–92. 1922.

28. Lasnier, M. E. Recherches biologiques sur deux Glœosporium. Bul. Soc. Myc. de France **24**: 17–42. 1908.

29. Laubert, R. Die Glœosporiumfäule der Banane und die Glœosporium und Phyllosticta – Blattfleckenkrankheit des Efeus. Gartenflora **59**: 409–415. 1910.

30. Leach, J. G. The parasitism of *Colletotrichum lindemuthianum*. Univ. Minn. Agric. Exp. Sta. Tech. Bul. **14**. 1923.

31. Saccardo, P. A. *Glœosporium Musarum* Cooke et Massee, Syll. Fung. **10**: 461. 1892.

32. ——— ———. *Glœosporium Musarum* Cooke et Mass., var. *importatum* Laubert. Syll. Fung. **22**: 1189–1190.

33. Savelli, Mariano. Studio critico sullo sviluppo del *Glœosporium Musarum* (Cooke et Mass.) Annalli della Real Accademia d'Agricoltura di Torino **57**: 184–192. 1914.

34. Shear, C. L. and Wood, Anna K. Studies on Fungous Parasites Belonging to the Genus Glomerella. Bu. of Plant Industry Bul. **252**. 1913.

35. Sheldon, J. L. The Ripe Rot or Mummy Disease of Guavas. W. Va. Univ. Agric. Exp. Sta. Bu. **104**. 1906.

36. Sherbakoff, C. D. Fusaria of Potatoes. N. Y. (Cornell) Agric. Exp. Sta. Memoir **6**. 1915.

37. Stackman, E. C. and Piemeisel, F. J. Biologic forms of Puccinia graminis on cereals and grasses. Journ. Agric. Res. **10**: 429–495. 1917.

38. Stevenson, John A. A check list of Porto Rican fungi and a Host Index. Journ. of the Dept. of Agric. & Labor. **2**: 205. 1918.

39. Stoneman, Bertha. A comparative study of the development of some anthracnoses. Bot. Gaz. **26**: 69–120, 1898.

40. Thom, Charles. Cultural studies of species of Penicillium. U. S. Dept. of Agric. B. A. I. Bul. **119**, 1910.
41. Voglino, Piero. I funghi parassiti delle piante osservati nella Providencia di Torina e regioni vicini nel 1913. Annalli della R. Accad. d'Agric. di Torino **57**: 171–172, 1914.

EXPLANATION OF PLATES

Figure 1

Graph showing difference in spore measurements of *Gloeosporium Musarum* in nature and in culture medium. A is strain X in nature. B same strain in potato medium.

Plate I

Fig. 2.—Typical discoloration of the peel of matured giant variety of banana produced by infection by *Gloeosporium Musarum*.

Fig. 3.—A "hand" of the dwarf variety of banana inoculated and kept in a moist chamber for ten days. Notice that the disease has worked from the base of each individual fruit toward the blossom end.

Plate II

Fig. 4.—Strain I of *Gloeosporium Musarum* after growing for ten days on Cook's No. II medium.

Fig. 5.—The same strain, the same age, on ripe banana peel medium.

Plate III

Fig. 6.—Strain III of *Gloeosporium Musarum*, eight days old, on potato medium.

Fig. 7.—Strain I on same medium, eight days old.

Plate IV

Figs. 8–12.—Spores of *Gloeosporium Musarum* from different varieties of bananas.

Fig. 13.—Spores from same source as Fig. 12, grown on potato medium.

Fig. 14.—Setae encountered in one of the cultures.

Fig. 15.—Spores of the fungus produced on conidiophores.

Fig. 16.—Typical sporodochia produced on culture medium.

Fig. 17.—Sunken, black pycnidia produced on culture medium.

Fig. 18.—Showing method of production and discharge of spores by the fungus.

Fig. 19.—Germinating spores showing the formation of appresoria.

Fig. 20.—Germinating spores without the formation of appresoria.

Fig. 21.—Germinating spores showing chemotrophic response influenced by the presence of a piece of the host tissue.

Fig. 22.—A spore twelve hours after germination.

Fig. 23.—A piece of the fungus mycelium.

Fig. 24.—A sub-epidermal cell showing penetration of the fungous hyphae.

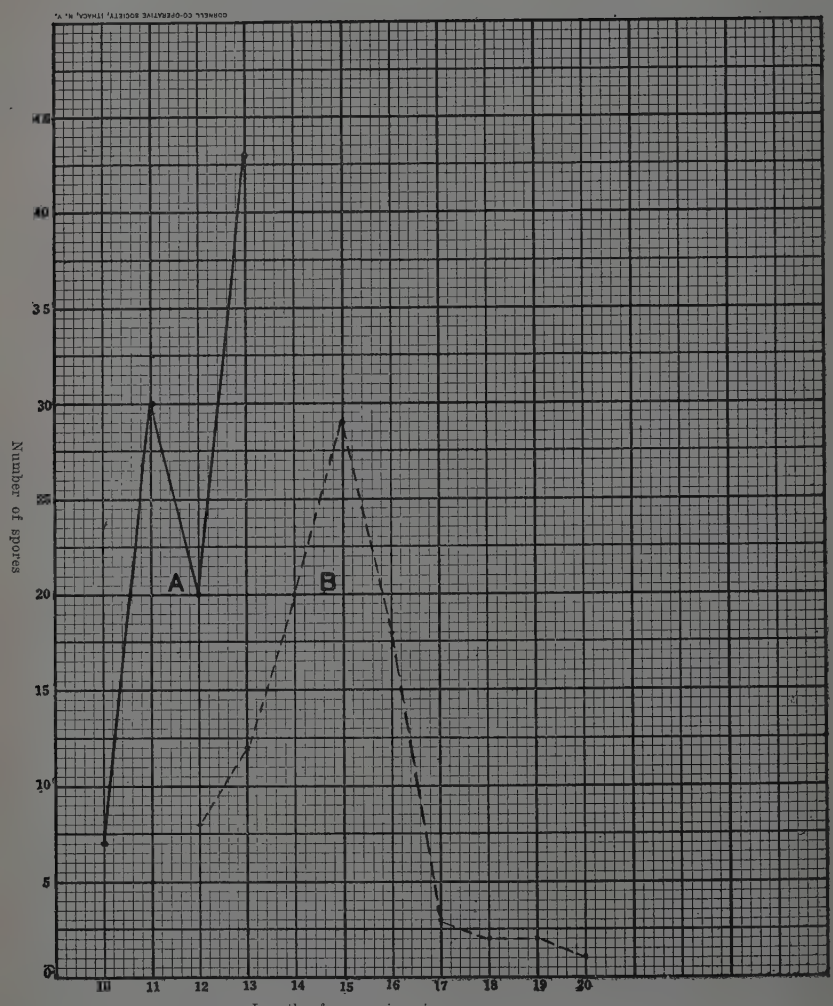
CORNELL CO-OPERATIVE SOCIETY, ITHACA, N. Y.
40
35
30
25
20
15
10
5
0
A
B
Number of spores
10
11
12
13
14
15
16
17
18
19
20
Length of spores in microns

FIGURE 1

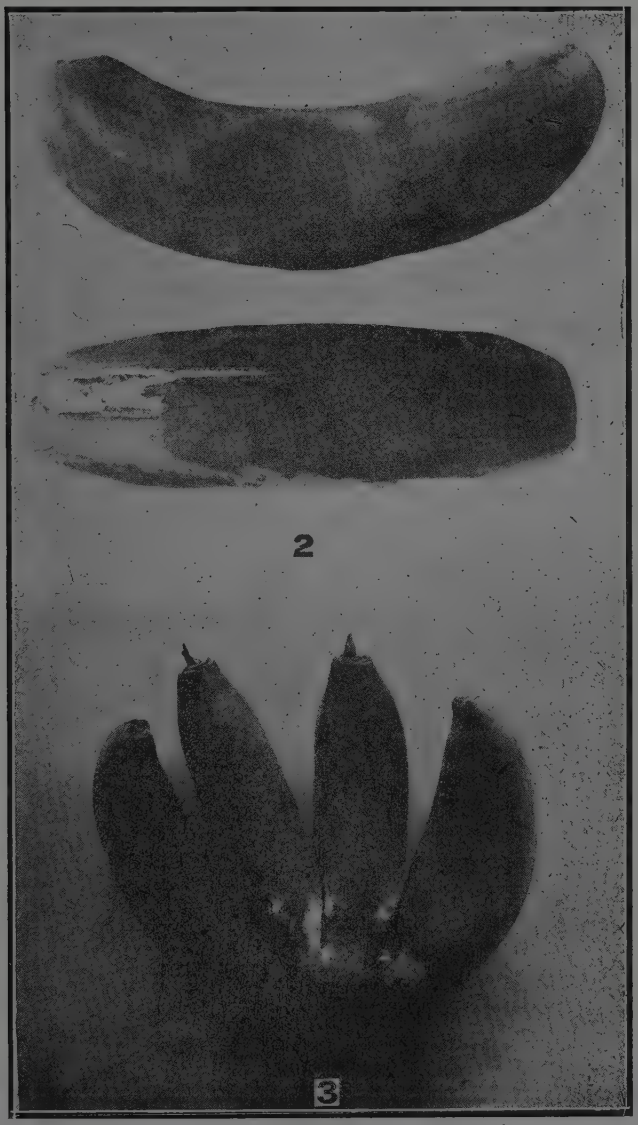

PLATE I

PLATE II

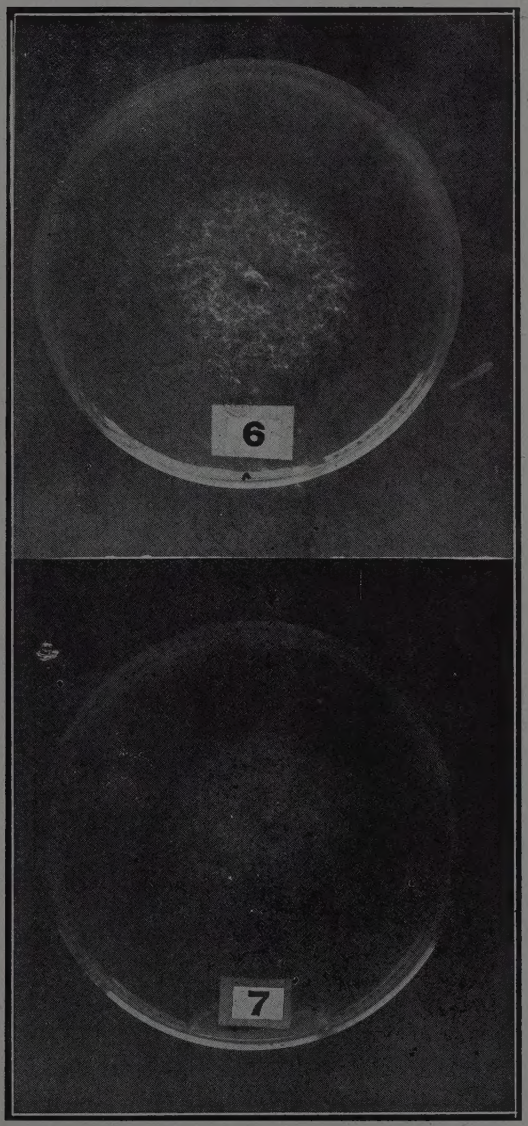

PLATE III

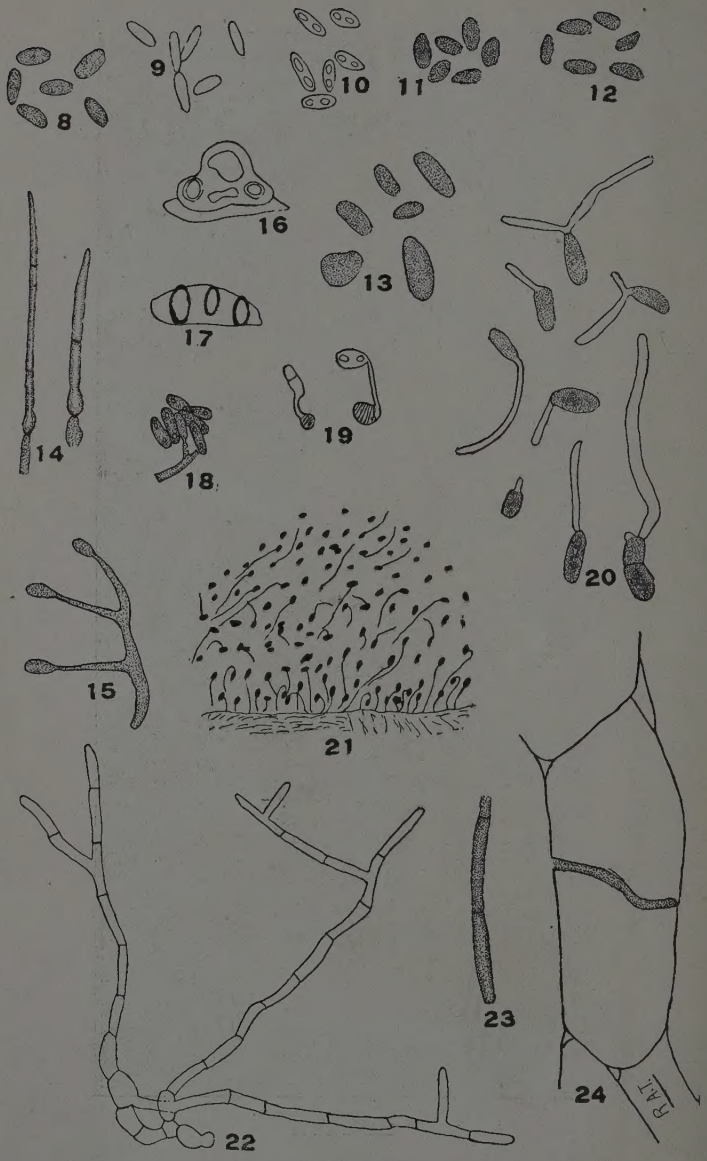

PLATE IV